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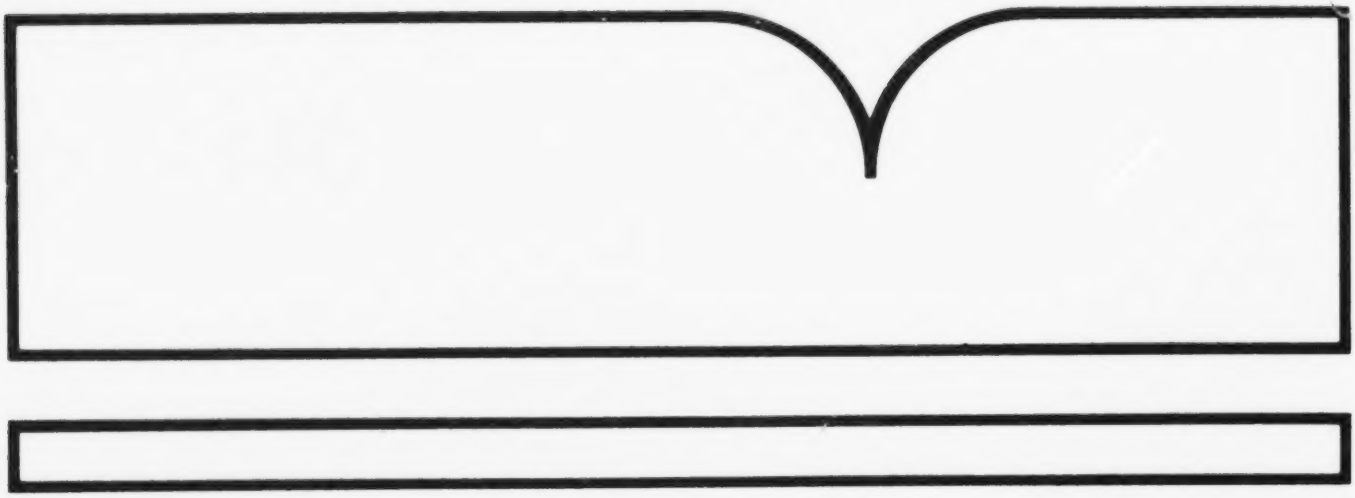
Modeling the Response of Greenhouse-Grown Radish Plants to Acidic Rain

Argonne National Lab., IL

Prepared for

Corvallis Environmental Research Lab., OR

1985



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16. ABSTRACT
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MODELING THE RESPONSE OF GREENHOUSE-GROWN RADISH PLANTS TO ACIDIC RAIN

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INTRODUCTION

CURRENTLY there is considerable interest in performing assessments to estimate vegetative losses from present and potential levels of acidity in precipitation. The research methodologies required to provide dose-response functions and damage estimates for crops and natural vegetation, however, are complex.[5,7] Because research materials and methodology vary among studies, comparisons are difficult and generalized conclusions regarding acidic rain impacts may be inaccurate.[6] Extrapolation of data from greenhouse experiments to estimate field effects or from field experiments to predict regional impacts has been used for economic assessments of air pollution impacts. However, additional research is necessary to verify the accuracy of such applications for estimation of acidic rain impacts.

The term 'acidic rain' which is used to describe wet deposition that has been affected by combustion emission products, is misleading in that it suggests a single pollutant, or variable. Analysis of the ionic composition of precipitation reveals a number of variables which may have important effects on vegetation. Those constituents which have the greatest potential in eliciting plant response are sulfate (SO_4^{-2}), nitrate (NO_3^-) and hydrogen (H^+) ion. Since sulfur and nitrogen nutrition in plants is interrelated, the concentrations and ratios of SO_4^{-2} and NO_3^- in combination with acidity may together determine plant

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response to rain. Stage of development and length of time between rain exposures have also been observed to affect radish response to simulated acidic rain.[8]

The following three greenhouse studies of radish were performed to: (1) determine physiological and productivity responses of the plants to various concentrations and ratios of acids in simulated rain in order to evaluate linear and non-linear response models, and (2) evaluate possible theories of the mechanism(s) of acidic rain impacts on radish in order to understand the differences in response between plants grown in the field with those grown in a greenhouse.*

MATERIALS AND METHODS

The experiments were performed in replicate during the autumn of 1981 and the spring of 1983 in a temperature and humidity-controlled greenhouse in non-filtered air. Radish seeds (cv. Cherry Belle) were planted in potting medium consisting of nutrients, trace minerals and a mixture of peat moss, vermiculite and perlite in a 3:1:1 ratio (by volume), in 10 × 10 cm plastic pots and later thinned to one per pot. The potting mixture was mounded above the pot and covered with plastic so that stem flow to the soil was almost nil. To allow free air–soil interchange while eliminating rain from the soil surface, the plastic covering was larger than the diameter of the pot and was not completely sealed around the pot rim. Thus, only the foliage was exposed to simulated rain treatments. Pots were irrigated from below with deionized water to saturation before planting and as needed during the experiment.

Experimental design consisted of eight treatments with 10 pots per treatment. Pots were grouped on the greenhouse bench according to replicate number and not by treatment. On the sixth day after planting the plants from each group were exposed to one of eight acidity levels in simulated rain treatments. Treatments with simulated rain took place 10 times during the 28-day period between planting and harvest. The length of time between simulated rain events consisted of 2 or 3 days. To achieve a typical rate for a convective rainfall, each 1-hr rain event was divided into 10, 6-min on–off periods, producing a rate of 0.76 cm/hr. To assure equal deposition of rain simulant from the nozzles, the pots were placed in a circle on a turntable at a speed of 1 rpm. After each rain event, pots were returned to a position on the greenhouse bench which was determined through a rotational scheme so that possible differences in microclimate along the bench would be equalized among the 10 groups.

Chemical composition for the eight rain simulants was based on the weighted average ion concentrations for NADP (National Atmospheric Deposition Program) sites in New York (sites 330860 and 332020), Pennsylvania (sites 392940 and 394200), and Ohio (sites 361760, 364900 and 367160) for the period 1 June–30 September 1979 (Table 1).† The weighted average hydrogen ion concentration for those sites was 0.065 mg/l; four of the rain simulants had higher acidities and four had lower acidities than the average (Table 1). Inorganic salts and sulfuric and nitric acids were added to deionized water to produce the desired acidity and chemistry for each rain treatment. The control rain simulant consisted only of inorganic salts with no strong acids. The sulfate to nitrate ratio (by weight) for the NADP sites was 2.37 and this ratio was maintained for each rain simulant. Rain simulants were applied to the plants by spraying upward through a stainless steel Spraying Systems Company 1/8 G1 full jet nozzle which produced a median drop volume of 1.1 mm. Quality assurance procedures included pH and conductivity measurements of samples from the batch solutions of each rain simulant prior to each event and on samples collected during the event.

Greenhouse conditions were roughly controlled to approximate normal diurnal fluctuations.

* The research reported in this paper was part of a larger interlaboratory comparison study which will be described in a future manuscript.

† National Atmospheric Deposition Program Report: Precipitation Chemistry 1979. Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, Colorado.

Table 1. Ion concentrations (mg/l) for ambient and simulated rain

pH	H^+	SO_4^{-2}	NO_3^-	Cl^-	Ca^{+2}	Mg^{+2}	K^+	Na^+	NH_4^+
4.19*	0.065	3.836	1.618	0.107	0.165	0.035	0.031	0.169	0.258
5.4	0.0025	1.114	0.457						
5.0	0.010	1.476	0.610						
4.6	0.025	2.015	0.839						
4.2	0.063	3.422	1.406		All simulants have these ions in the same				
3.8	0.158	6.737	2.841		concentrations as ambient (pH 4.19) rain.				
3.4	0.398	15.239	6.444						
3.0	1.000	36.591	15.495						
2.6	2.512	90.232	38.233						

*Ambient rain: weighted average concentrations for New York, Pennsylvania, and Ohio NADP sites, 1 June–30 September 1979.

Temperature ranged between 18°C (night) and 30°C (day) and averaged 26°C in 1981 and 23°C in 1982. Relative humidity ranged from 30% (day) to 80% (night) and averaged 42% in 1981 and 38% in 1983. A combination of high pressure sodium and metal halide vapor lights was used to extend the photoperiod of 15 hr. These lights provided a photon flux density of 380 $\mu mol/m^2/sec$.

Each plant was evaluated (subjectively) for presence and extent of visible injury symptoms prior to each simulated rain event. The radishes were harvested on day 28 after planting and measured immediately to determine fresh mass. Roots and shoots were weighed again after drying to constant weight at 68°C, for dry mass determinations.

Just prior to harvesting plants from the second experiment, net photosynthetic rates of the fourth oldest true leaf of each of nine plants from each treatment were determined through measurements of CO_2 uptake. This was done by placing the leaf, immediately after detachment from the plant, in a sealed plexiglass chamber where air was circulated by means of a small fan. The difference in CO_2 content of two air samples removed from the chamber with a 10-cc syringe, one at 10 sec and one at 30 sec after sealing the leaf in the chamber, was determined through infrared gas analysis (ANARAD AR-500 series CO_2 analyzer). Surface area and dry weight were determined for each of the sampled leaves and photosynthesis was calculated on the basis of CO_2 uptake per leaf area and per plant.

An additional experiment was performed simultaneously with the dose-response study in 1983 to determine whether the ratio of SO_4^{-2} to NO_3^- in rain affects the response of radish to acidic rain. Plant growth and culture conditions for the two experiments were identical. There were 10 plants per treatment and four treatment groups consisting of a control rain (no strong acids) and acidic rain having one of three different $SO_4^{-2}:NO_3^-$ ratios (Table 2). Concentrations of background ions (non-acidic salts) and total deposition of rain were identical for all experiments. Net photosynthesis was determined for each plant prior to

Table 2. Concentration and ratio of hydrogen ion and acid anions in simulated rain

	H^+	SO_4^{-2}	NO_3^-	
pH		(mg/l)		$SO_4^{-2}:NO_3^-$
5.4	0.0025	1.11*	0.47*	
3.0	1.00	38.17	12.72	3:1
3.0	1.00	34.62	17.31	2:1
3.0	1.00	27.07	27.07	1:1

*The low concentrations of these ions in the control rain are from the addition of non-acidic salts (background ions).

harvest as in Experiment 2. Wet and dry weights of plant parts were measured after the 28-day growth period.

Statistical analyses were performed using the Statistical Analysis System (SAS) software with an IBM 3033 VM/370 computer.

RESULTS

Injury symptoms

Visible injury, in the form of leaf necrosis, was observed only in treatment groups which received simulated rain with a pH of 3.4, 3.0, and 2.6, and percent injured tissue increased as the acidity of the rain simulant increased. Within a treatment group, leaves exposed to the same number of rain events had a similar degree of injury, regardless of plant age. At the time of harvest, injury was greatest in the cotyledons and oldest true leaves, which had been exposed to the greatest number of rainfall events. In the plants treated with the pH 2.6 rain simulant, necrotic lesions covered up to 50% of the surfaces of oldest leaves; youngest leaves were only slightly injured, but were reduced in size. Leaves of plants subjected to the highest acidity levels resembled those of drought-stressed plants with lower surface area to dry weight ratios. These results are similar to those of other greenhouse studies of radish[3,10] but differ from field-grown radish which exhibit little, if any, visible injury from simulated acidic rain.[3,12]

Yield

Although radish weights were lower in the second experiment compared to the first, the overall response to the simulated rain treatments was approximately the same (Fig. 1). The lower yield in the second experiment was probably the result of slightly lower average temperature and humidity (i.e. the plants in Experiment 1 grew under conditions where temperatures averaged 3°C higher and humidity was 4% higher than in Experiment 2). Since each experiment can be considered a trial, yield data were transformed to percent of control so that results from both experiments could be compared and combined. Analysis of variance (ANOVA) of the combined data indicates a significant treatment effect ($P < 0.0001$) on radish yield (Table 3). Application of a simple linear model to the pooled data from both experiments (Table 3) indicates that the marketable yield (root and hypocotyl) of radish is significantly affected by the hydrogen ion concentration of simulated rain. Parameter estimates for the model indicate that the relationship

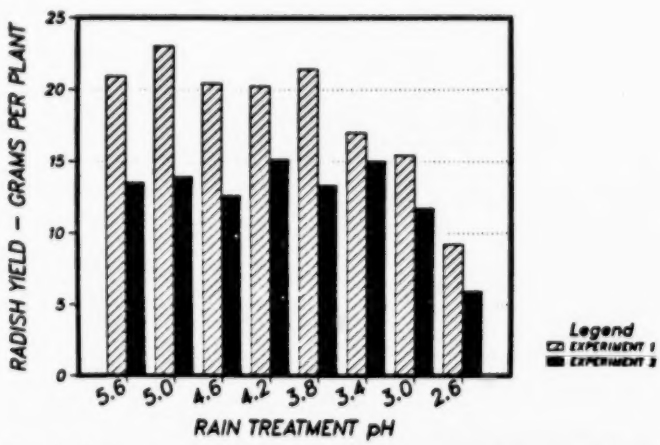

FIG. 1. Market yield (root and hypocotyl) of radish vs rain acidity. Results are means for two experiments, $\eta = 10$.

Table 3. Analysis of variance (ANOVA) and comparison of models describing radish yield (as percent of control) as a function of hydrogen ion concentration in simulated rain

Source	d.f.	MS	LOF *F* value	Model *F* value	R^2
ANOVA					
Treatment (H^+)	7	0.845		21.29***	0.495
Error	152	0.04			
Corrected total	159	0.075			
Model					
Linear	1	5.745	0.717	144.7***	0.481
Quadratic	2	2.877	0.810	72.5***	0.482
Cubic	3	1.894	1.473	47.7***	0.475
Plateau-linear	2	2.886	0.728	72.7***	0.483

d.f. = degrees of freedom, SS = sum of squares, MS = mean square, LOF (MS) = lack of fit (model) = trt SS—model SS/trt d.f.—model d.f., *F* (treatment) = ANOVA MS ÷ ANOVA error MS, *F* (model) = model MS ÷ ANOVA error MS, *F* (LOF) = LOF MS ÷ ANOVA error MS, R^2 (treatment) = ANOVA SS ÷ ANOVA corr. total SS, R^2 (model) = model SS ÷ ANOVA corr. total SS.
Significant at ** $P = 0.05$, *** $P = 0.0001$.

between simulated rain acidity and yield is negative.

Addition of quadratic and cubic terms does not improve the fit of the model (Table 3). Application of a log function, through use of pH rather than hydrogen ion concentration as the source of variation, also does not produce a better fit model (Table 4). Although the log (pH) relationships are statistically significant, the Lack of Fit (LOF) statistic[1] indicates that residual differences are also significant and that the linear and quadratic log models are not an adequate description of the data.

The effect of the two highest acidity levels on the shape and slope of the response curve is more readily observed when the data are expressed on the basis of hydrogen ion concentration rather than pH (Fig. 2). When these two points (pH ≤ 3.0) are eliminated from the data set for Experiment 1, analysis of variance indicates no acidity effect. When the three highest acidity levels (pH ≤ 3.4) are excluded from the analysis

Table 4. Analysis of variance and comparison of models describing radish yield (as percent of control) as a function of pH of simulated rain

Source	d.f.	MS	LOF *F* value	Model *F* value	R^2
ANOVA					
Treatment (pH)	7	0.845		21.29***	0.495
Error	152	0.04			
Corrected total	159				
Model					
Linear	1	3.139	11.66***	79.06***	0.429
Quadratic	2	2.56	16.90***	64.53***	0.429
Cubic	3	1.894	1.48	47.71***	0.475
Plateau-linear	2	2.862	0.965	71.34***	0.479

See Table 3 for explanation of symbols.

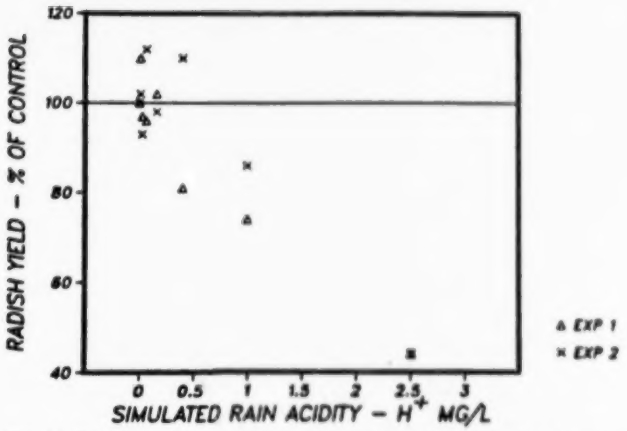

FIG. 2. Radish yield (as percent of control) vs hydrogen ion concentration of simulated rain. Data are means for two experiments, $\eta = 10$.

of variance for Experiment 2 there are no significant acidity effects. Accordingly, a plateau-linear model (equation 1) was applied to the data for the individual and combined experiments.

$$y = \beta_1 + t \quad [\beta_2(H^+ - \theta)], \qquad (1)$$

where y is the radish market yield as % of control, $t = 1$ when $H^+ > \theta$ and 0 when $H^+ \leq \theta$, H^+ is mg/l H ion in simulated rain and θ is the threshold H^+ concentration.

The parameter estimate for theta (θ) (threshold concentration) varies considerably between the two experiments (Table 5). The plateau-linear model of the data indicates that all acidity levels caused negative effects on radish yield in the first experiment while only the two highest levels reduced yields in the second experiment. Analysis of the combined data indicates a threshold pH of 3.86. Thus the linear model appears more appropriate for describing Experiment 1 while the plateau-linear model may be best for Experiment 2 and for the combined data, although elimination of the highest acidity levels from data tested by ANOVA suggests a threshold response for both experiments.

Yield results from Experiment 3 revealed no significant treatment effects on shoot weight; however, root and hypocotyl yield were significantly ($P = 0.05$) affected by acidity and by the SO_4^{-2}:NO_3^- ratio of acidic simulant (Fig. 3). The data suggest that nitrate levels in acidic rain may have a greater impact on below-ground radish growth than sulfate levels since the 2:1 and 1:1 SO_4^{-2}:NO_3^- acidic rain treatments significantly reduced yield while the 3:1 treatment was not significantly lower than the non-acidic control.

Photosynthesis

The photosynthetic rate, when expressed as CO_2 uptake on the basis of leaf area, was negatively correlated with shoot dry weight (corr.

Table 5. Parameter estimates determined by plateau-linear model for acidic rain effects on radish.
Yield $= \beta_1 + t[\beta_2(H^+ - \theta)]$

	Threshold estimate – θ			
Experiment	H^+/mg/l	pH	β_1	β_2
1	0.0025	5.60	100	−0.235
2	0.4277	3.37	103	−0.283
Combined	0.1377	3.86	101	−0.243

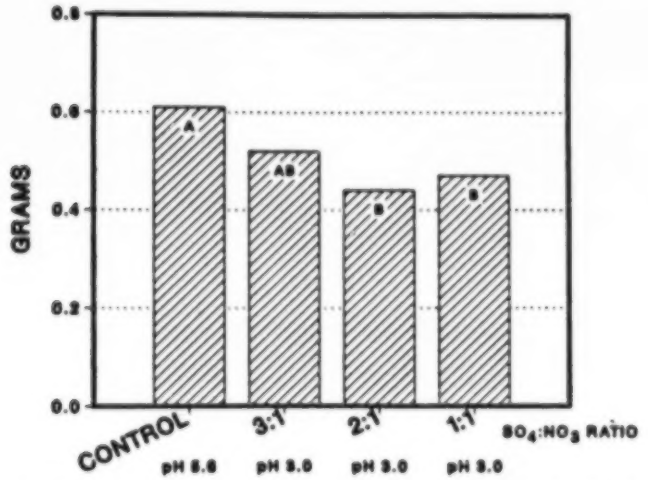

FIG. 3. Market yield of radish vs the sulfate: nitrate ratio in simulated acidic rain, $\eta = 10$. Means with a common letter are not significantly different according to Duncan's Multiple Range Test, $P \leq 0.05$.

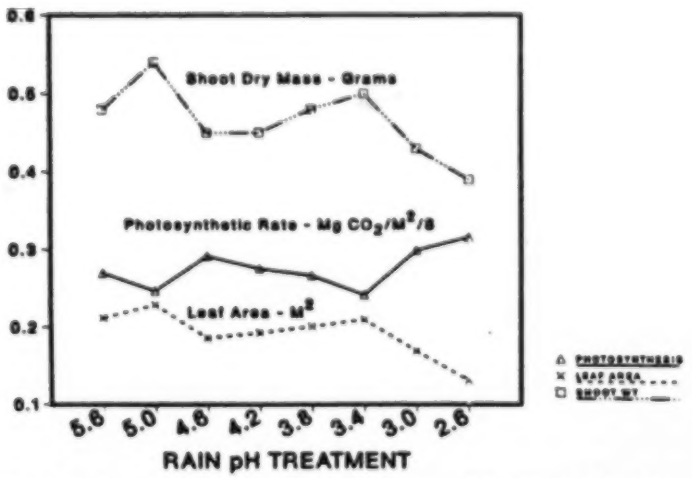

FIG. 4. Leaf area per plant, photosynthetic rate per unit leaf area and shoot dry mass of radishes exposed to simulated rain.

Table 6. Analysis of variance for treatment (H^+ concentration in rain) effects on photosynthetic rate

Source	d.f.	SS	MS	*F* value	R^2
ANOVA					
Treatment (H^+)	7	0.0388	0.0055	1.38	0.133
Error	63	0.2539	0.004		
Corrected total	70	0.2927			

See Table 3 for explanation of symbols.

coef. = −0.4, $P = 0.0002$) and leaf area per plant (corr. coef. = −0.5, $P = 0.0001$) (Fig. 4). Analysis of variance of these data indicates no significant treatment (acidity) effects on photosynthetic rate per unit leaf area (Table 6). However, photosynthetic rate expressed on a per plant basis, decreases with increasing acidity (Fig. 5), because shoot area and biomass decrease, especially at the two highest acidity levels. Thus, the higher rate of CO_2 uptake per unit leaf area in plants treated with the highest levels of rain acidity was not enough to offset the effect of the reduced leaf area and injured tissue on photosynthetic rate per plant. Application of the data to a linear model indicates a significant negative relationship between hydrogen ion concentration in rain and CO_2 uptake per second per plant (Table 7). The correlation of total leaf area per plant with acidity is also demonstrated by analysis of variance and a linear model (Table 8). These results reveal the importance of expressing photosynthetic rate not only on an area basis but also as a function of the entire plant in order to define completely the response.

Results from Experiment 3 indicate that photosynthetic rates were unaffected by the

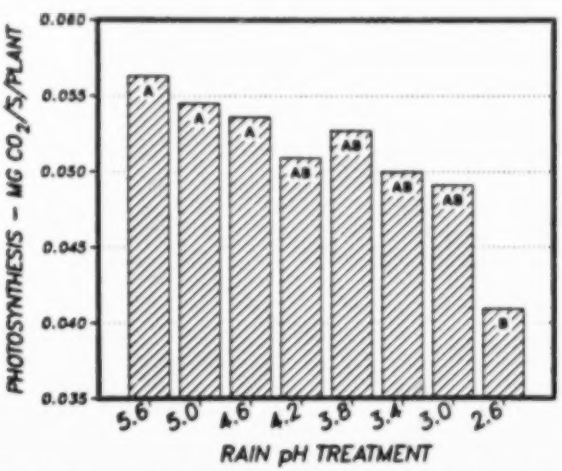

FIG. 5. Photosynthetic rate per radish plant vs simulated rain pH. Bars with a common letter are not significantly different ($P > 0.05$) according to Duncan's Multiple Range Test, $\eta = 9$.

Table 7. Model test for treatment (H^+ concentration in rain) effects on plant photosynthetic rate. Model tested: mg CO_2/plant/sec = mg/l H^+

Source	d.f.	SS	MS	*F* value	R^2
Model	1	0.00115	0.00115	9.76**	0.124
Error	69	0.00815	0.00012		
Corrected total	70	0.000931			
LOF	6	0.00162	0.00027	0.21	

See Table 3 for explanation of symbols.

SO_4^{-2}:NO_3^- ratio in radish plants treated with rain having a pH value of 3.0.

DISCUSSION

This study demonstrates the value of repeating an experiment to obtain a better characterization of experimental variability due to non-controlled factors. Combining data from two or more such experiments allows a general or average assessment of response; however, an examination of differences in experimental conditions that may have influenced experimental results from year to year may lead to a better understanding of the mechanisms of response. Results from these experiments and others (unpublished) from our laboratory, suggest that simulated acidic rain is more damaging to plants growing in ideal conditions as opposed to those which undergo some stress. The radishes in Experiment 1, which grew in conditions of higher temperature and humidity, had a higher yield than those in Experiment 2, but were apparently also more sensitive to simulated acidic rain. A comparison of acidic rain studies performed on radish in the field (where plants are subjected to other environmental stresses) compared to those performed in a controlled environment indicate that the field-grown plants are insensitive to simulated acidic rain (Table 9). Also, radish cultivars appear to have differing sensitivities. For these reasons, extrapolation of results from greenhouse studies for damage assessments should not be done unless the results are verified by field experiments.

Although all the yield models tested using the combined data from this study were statistically significant ($P < 0.0001$) it is obvious that some do not provide an accurate assessment of the experimental results (Fig. 6). For example, a linear regression of pH vs radish yield (combined data)

Table 8. Analysis of variance and model test for treatment (H^+ concentration in rain) effects on leaf area per plant. Model tested: cm^2 = mg/l H^+

Source	d.f.	SS	MS	*F* value	R^2
ANOVA					
Treatment H^+	7	5418060	774008	3.86**	0.300
Error	63	12628041	200445		
Corrected total	70	18046100			
Model	1	4229084	4229084	21.10***	0.234
Error	69	13817017	200247		
Corrected total	70	18046101			
LOF	6	1188976	198163	0.989	

See Table 3 for explanation of symbols.

Table 9. Comparison of studies of simulated acidic rain effects on radish

Source	Cultivar	Rainfall amt (cm)	Effect*	Location
Lee and Neely[9]	Cherry Belle	12	0	Field
Troiano *et al.*[12]	Champion	5	+	Field
Troiano *et al.*[12]	Champion	6	+	Field
Evans *et al.*[2]	Cherry Belle	0.32	0	Field
Lee *et al.*[10]	Cherry Belle	12	—	Controlled envir.
Evans *et al.*[3]	Cherry Belle	7.6	—	Controlled envir.
Irving (this paper)	Cherry Belle	7.6	—	Controlled envir.

*Effect on marketable yield from rainfall at pH of 3.0 compared to 5.6; 0 = no effect, + = positive effect, — = negative effect.

is statistically significant ($P < 0.0001$) and predicts that current levels of acidity in rain (pH ~4.1) reduce radish yield by 20% compared to rain having no strong acids (pH ~ 5.6), even though examination of the actual experimental data suggests little, if any, impact. Analysis of residuals, through determination of the Lack of Fit (LOF) statistic should result in rejection of this model however (Table 4). Even the linear H^+ model would predict impacts from ambient acid rain, while the plateau-linear model of H^+ vs radish yield predicts no yield reductions from acidity in ambient rain (Fig. 6). The models presented in this paper are not intended for use in assessments of acidic rain impacts, but rather are examples of the range of interpretations possible in evaluating acidic rain effects. Evans *et al.*,[3] also performed a dose-response study of greenhouse-

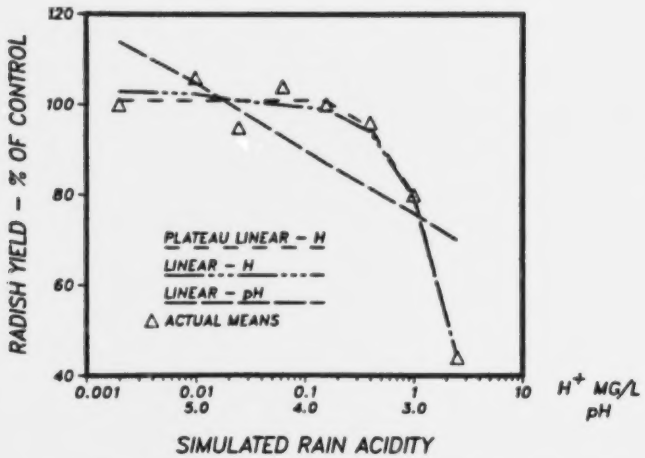

Fig. 6. Market yield of radish as a function of rain acidity. Actual means and functions from three models (plateau linear, linear, and log linear) are presented.

grown radish exposed to acidic rain, although they did not generate a dose-response function. They reported that marketable yield from plants treated with the two highest acidity levels (pH 3.0 and 2.6) were significantly lower than yields at pH 3.4, 4.2, 4.6 and 5.6. There appeared to be a plateau effect between pH levels of 4.6 and 3.4 and a decrease in yield between 5.6 and 4.6. Although their response curve would be somewhat different than that produced in this study, their results support the hypothesis that acidic rain effects on radish are non-linear. Other groups assessing air pollution impacts use non-linear models to describe crop response.[4]

The results from Experiment 3 indicate that plants respond to the sulfate and nitrate components of acidic rain in addition to hydrogen ion. This information is needed for a complete evaluation when modeling plant response to rain acidity since the SO_4:NO_3 ratio varies on a regional basis in the United States and can be expected to change under different scenarios of energy production and emission restrictions.

The absence of a treatment effect on photosynthetic rate per unit leaf area in this study indicates that the effect of acid rain on harvested yield was not due to a direct effect on photosynthesis, although periodic changes in photosynthetic rate at certain times following a rain event may not have been measured. The reduced leaf area in plants treated with acidic rain in this study was due to necrotic injury as well as decreased leaf expansion. The observed reductions in photosynthetically active tissue at the highest acidity levels in this study would result in less photosynthate available for allocation to the root and hypocotyl, thus resulting in decreased yield.

A key to understanding the mechanisms of acidic rain impacts on radish yield may be to determine causes for reduced leaf expansion. Perhaps carbohydrate reserves are used for repair and recovery processes rather than growth processes in the acidic rain-stressed plants. The existence of recovery processes in radish plants exposed to acidic rain is suggested by results of experiments which indicated increased tolerance to acidity in response to an increase in the length of rain-free intervals between acidic rain exposures.[8] Additionally, meristematic cells of young leaves may be injured by the high acidity treatments, resulting in decreased cell division, and thus reduced size.

The greater effect of nitric compared to sulfuric acid suggests that nitrate ions are more effective in leaf surface exchange reactions than sulfate ions. Field-grown plants may be more resistant to the direct foliar effects of acidic rain because of greater development of leaf waxes and cutin, known to occur in plants exposed to stresses such as wind and drought.[11]

CONCLUSION

When applied to the foliage only, there appeared to be a threshold for negative effects on radish yield from acidity in rain. The threshold pH level may have been dependent on environmental conditions during the experiment. Significant negative effects on marketable yield of radish from simulated rain occurred at pH levels below 3.8 in one dose-response experiment and below 3.4 in another experiment where the temperature, humidity, and overall growth rates were also lower.

The concentrations and ratios of SO_4^{-2} and NO_3^- together with acidity determined the response of radish to simulated acidic rain. Nitric acid in rain appears to have a greater effect than sulfuric acid in reducing radish yield at pH 3.0.

Acid rain had no effect on photosynthetic rate per unit leaf area when measured prior to harvest; however, total plant photosynthesis decreased with increasing acidity because leaf area per plant was reduced in the highest acidity treatments as a result of direct foliar injury and decreased leaf expansion. Reduced availability of photosynthate (because of a decrease in photosynthetically active tissue) was thus the most probable cause of the yield decrease at the highest acidity levels.

The use of extremely high levels of simulated rain acidity ($pH \leqslant 3.0$) in controlled studies can obscure effects at more moderate acidity levels and have an overwhelming influence on the outcome of linear dose-response functions used to describe the results. Evaluation of a number of mathematical relationships is advised when attempting to model the biological response to acidic rain. Multivariate relationships should be established since a number of variables, in ad-

dition to acidity, have been found to affect plant response to rain acidity.

Negative effects on radish yield from acidic rain have been reported for this and other greenhouse experiments, but have never been observed in field studies. This may be due to the possibility that plants growing under more favorable conditions (such as in a greenhouse or as in Experiment 1 compared to Experiment 2) are more susceptible to direct injury from acidic rain than plants exposed to other environmental stresses (such as in the field) because leaf surface characteristics may differ between the two groups of plants.

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